EFFECTS OF *XANTHIUM* LEAF EXTRACTS ON LETTUCE SEED GERMINATION[1, 2]

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ABSTRACT

Seed germination in lettuce var. Grand Rapids, and floral induction in *Xanthium* are phytochrome-mediated processes. The pigment conversion which triggers these two rather diverse developmental events appears to be essentially the same. The present work was conducted to test the possibility that secondary metabolic steps following the initial triggering phase might also be shared by these two morphological processes. Aqueous extracts were made from leaves of vegetative *Xanthium* plants, as well as from those having received one or five floral inductive photoperiods. These extracts were applied to lettuce seeds to test for their possible promotive or inhibitory effects on germination. No promotive effect on germination could be demonstrated. Extracts from vegetative *Xanthium* plants had a marked inhibiting effect on germination of lettuce seeds. This inhibitory effect could be substantially reduced if the *Xanthium* plants were given floral-inductive treatments prior to extraction. Thus, as *Xanthium* leaves progress from non-inductive to inductive photoperiods, there appears to be a decrease in content of a germination-inhibiting substance, which parallels the generally accepted time period of flowering stimulus build-up.

Two of the better known phytochrome-mediated processes in plants are the photoperiodic response in flowering (Parker *et al.*, 1946; Vince, 1972) and the germination of light-sensitive seeds (Borthwick *et al.*, 1954; Flint and McAlister, 1935; Mohr, 1972; Rollin, 1972). It has been established that certain triggering events in flowering and seed germination can be traced to a phytochrome pigment conversion (Rollin, 1972; Vince, 1972).

A possibility exists that secondary metabolic steps subsequent to the initial light-triggered phase might be similar or even identical in the flowering and germination processes. McIlrath and Bogorad (1958) studied this possibility in their work involving the flowering of the short-day plant, *Xanthium*, and the germination of light-sensitive lettuce seeds (var. Grand Rapids). They were able to show that lettuce seeds in petioles of floral-induced *Xanthium* leaves yielded a higher germination percentage than seeds implanted in petioles of vegetative leaves. In no case, however, could these workers get germination percentages which were comparable to petri-plate-grown water controls. Although the lack of high germination percentages could be attributed to the presence of a germination inhibitor, there was some question that the physical aspects of the implantation method itself could in part be responsible for lack of high germination percentages.

In an attempt to circumvent some of the problems involved in the implantation method and to extend previous findings, the present work was conducted using extracts from *Xanthium* leaves applied to lettuce seeds. Tests were conducted to determine if floral-induced *Xanthium* leaves produce a substance which promotes lettuce-seed germination or whether non-induced leaves produce an inhibitor of lettuce-seed germination. Lettuce seeds pre-treated with far-red light or retained in the dark were used in testing extracts for promotive effects on germination, while seeds pre-treated with red light were used in testing extracts for inhibitory effects on germination.

METHODS AND MATERIALS

Seeds of lettuce (*Lactuca sativa* var. Grand Rapids) obtained from Livingston Garden Seeds, Columbus, Ohio (Lot no. 778) were used in all germination tests conducted. Test plates con-

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sisted of 100 seeds spread over one sheet of 7 cm Whatman #1 filter paper wetted with 3 ml of the solution to be tested, placed within a sterile plastic disposable petriplate. After an initial 90-minute imbibition period, seeds receiving a light treatment were irradiated with either 15 minutes of red or 15 minutes of far-red light. Seed plates having received a light treatment, as well as those receiving only a dark treatment, were wrapped in aluminum foil prior to their being transferred to an incubating cabinet at 26°C. Seed counts were made after 48 hours. A seed was considered germinated if the white radicle had penetrated the seed (achene) coat.

The red-light source for seed treatment was obtained by passing fluorescent light through a 30-cm-square Carolina Biological Supply (CBS) red 650 light filter. A far-red light source was obtained by passing incandescent light through a 7-cm-thick heat-absorbing water layer in addition to a 30 cm square CBS far-red 750 light filter.

Xanthium pensylvanicum Wallr. plants used as the source of leaf extracts were raised from small seedling stage to maturity using hydroponic conditions in a controlled environment chamber with 18-hour photoperiods. Leaf extracts were made from plants about four to six weeks-of-age which were approximately 15 to 20 inches tall.

Using a single leaf induction system patterned after that of Searle (1961) it was found that *Xanthium* leaves with a mid-vein length of 7 to 9 cm were most effective in floral induction, so leaves of this size were subsequently used in making all *Xanthium* leaf extracts.

In preparing *Xanthium* extracts, leaf blades were removed, weighed, and fragmented with a razor. Leaf fragments were ground with a small quantity of washed quartz sand with mortar and pestle in a pH 7 phosphate buffer. A 10-g fresh-weight sample of leaf material was ground in 10 ml of buffer solution. The resulting slurry was centrifuged at low speed for 15 minutes to remove sand and cell debris. The cloudy, greenish colored supernatant was then centrifuged at 7,500 x G for 30 minutes. When decanted, the resultant supernatant was a clear brownish-amber color. This extract was diluted to 1/10 or 1/100 the original concentration before application to the lettuce seed test-plates.

Preliminary tests of the pH tolerance of lettuce seeds during germination indicated that lettuce-seed germination was nearly optimal over the range of pH 6 to pH 8. Because of these preliminary findings, it was decided to expand the original extracting procedure to include extracts made with buffers at pH 6 and pH 8 in addition to those at pH 7. It was felt that such expansion of the scope of the tests would facilitate the determination of whether midly basic or mildly acidic conditions modified the effectiveness of any *Xanthium* extracts on lettuce-seed germination.

Xanthium plants from which leaf extracts were to be made received one of three different photoperiodic treatments. One group of plants received no inductive short-day treatments and were retained on the 18-hour photoperiods. A second group of plants was raised under 18-hour photoperiods but was given one inductive 16-hour long-night prior to extraction. A third group raised on 18-hour photoperiods received five consecutive long-night treatments prior to extraction. The leaves were removed from the plants in each group approximately five hours into the light phase which immediately followed the conclusion of the photoperiodic treatment as indicated above.

Six germination plate replicates were made for each extract tested. Three control plates, using 3 ml of buffer at the same pH as test plates, but containing no *Xanthium* extract were included in each test.

EXPERIMENTATION AND RESULTS

Experiment 1. Dark-grown Grand Rapids lettuce seeds normally yield a very low germination percentage. Dark-grown lettuce seeds were treated with leaf extracts from *Xanthium* plants having received zero, one, or five inductive long-night treatments to determine if there was any promotive effect of such extracts on lettuce-seed germination. Extracts at three pH values (6, 7, 8) and at two concentrations, (1/10 and 1/100 original concentration) were used as explained above. The results of these tests are shown in table 1.

An evaluation of the data shown in table 1 indicates that there was no statistical significance in the variance between the average germination with any extract tested and its corresponding control. There was no statistical significance in average germination between the treatments with regard to the number of inductive cycles received by the leaves before the extract was made. Thus it appears that there was no promotion of lettuce seed germination by application of any of the *Xanthium* leaf extracts used in this experiment.

Experiment 2. In experiment 2 all tests of experiment 1 were repeated, except that seeds in each test plate received a 15 minute far-red light treatment following the initial 90 minute imbibition period. As with the dark germination test, far-red treated seeds normally yield low germination percentages and provide a second

TABLE 1

Percent germination of dark germinated lettuce seeds incubated 48 hours on Xanthium *leaf extracts*

Xanthium floral inductive cycles prior to leaf extract prep.	Extract Concentration Dilution		Control % Germination	pH
	1/10 original	1/100 original		
None	1.7	4.8	4.0	6
One	5.0	8.1	6.5	6
Five	0.7	3.0	3.0	6
None	1.1	0.8	2.0	7
One	2.8	1.8	4.0	7
Five	2.0	5.0	5.5	7
None	0.7	3.1	2.0	8
One	1.3	1.6	3.5	8
Five	2.3	6.6	6.5	8

means of checking for promotive effects of *Xanthium* leaf extracts. The results of the second experiment are shown in table 2.

As with the data of experiment 1, an analysis of variance on these data show no statistical significance to any deviation of average percent germination of controls and any test treatment shown in table 2. The data support the original indication that there is no promotion of germination by any extract tested.

TABLE 2

Percent germination of lettuce seeds treated with far red light for 15 minutes and incubated 48 hours on Xanthium *leaf extracts*

Xanthium floral inductive cycles prior to leaf extract prep.	Extract Concentration Dilution		Control % Germination	pH
	1/10 original	1/100 original		
None	0.5	0.7	1.0	6
One	1.7	4.1	3.2	6
Five	3.1	5.1	4.2	6
None	1.0	1.0	2.5	7
One	0.5	1.5	2.0	7
Five	1.3	3.8	4.7	7
None	0.3	0.5	2.0	8
One	0.5	1.0	0.9	8
Five	1.7	0.8	2.0	8

Experiment 3. Using a modification of the procedures used in experiment 2, a third experiment was conducted to determine if there was an inhibitory effect of any *Xanthium* leaf extracts on lettuce seed germination. In experiment 3 a 15 minute red light treatment was given to all test plates following an initial 90-minute imbibition period. The red light treatment normally yields high lettuce seed germination percentages, and would thus facilitate the determination of any

inhibitory effect the leaf extracts might have on germination. The results of experiment 3 are shown in table 3.

An analysis of variance using Duncan's New Multiple Range Test (Freund *et al.* 1960) indicated that there was significance at the 0.01 probability level regarding differences in average percent germination between tests of non-induced and induced (one-or five-day) treatments at each concentration and pH tested. The pH of the extract within the range tested was not a significant factor in this response. The same pattern of response was obtained within the tests at each of the three pH values studied.

TABLE 3

Percent germination of lettuce seeds treated with red light for 15 minutes and incubated 48 hours on Xanthium *leaf extracts*

Xanthium floral inductive cycles prior to leaf extract prep.	Extract Concentration Dilution		Control % Germination	pH
	1/10 original	1/100 original		
None	45.0	54.3	88.0	6
One	81.0	83.3	92.0	6
Five	85.7	93.7	93.0	6
None				
None	48.0	54.3	91.5	7
One	77.7	88.3	93.0	7
Five	84.7	92.0	91.5	7
None	49.3	66.3	83.0	8
One	71.3	81.3	78.5	8
Five	71.7	84.7	84.5	8

DISCUSSION

Xanthium is one of the few photoperiodic plants which can attain a threshold level of the floral stimulus sufficient to cause flowering after only one inductive long-night treatment. A further build up of the floral stimulus, leading to more rapid flowering, occurs if five consecutive inductive long-night treatments are given.

The data of the experiment summarized in table 3 support the conclusion that the germination inhibitor present in vegetative *Xanthium* leaves becomes significantly diminished when leaves are given one floral-inductive treatment, and is further diminished when the leaves are given five inductive treatments. Thus it appears that this decrease in inhibitor level parallels the increase in floral stimulus level in *Xanthium* leaves during floral induction. Although this parallel in the time of inhibitor level decrease and floral stimulus increase could indicate some biochemical interrelationship, the data presented do not rule out the possibility that these are concidental unrelated phenomena.

Xanthium leaves have been studied as a source of growth inhibiting substances for some time. Little *et al.* (1950) extracted a white crystalline substance from *Xanthium* which was given the name "xanthatin". This substance was found to have strong antibacterial and antifungal properties. Geissman *et al.* (1954) assigned the formula $C_{15}H_{18}O_3$ to xanthatin and also isolated a related compound from *Xanthium* which was given the name "xanthinin" and has the formula $C_{17}H_{22}O_5$. Further tests with xanthatin (Bonde, 1953; Bonde and Khudairi, 1954; Khudairi and Bonde, 1954) indicate that this compound can inhibit indole-acetic-acid induced growth in *Avena* coleoptiles. There was some indication

that the concentration of xanthatin may be slightly lower in floral-induced *Xanthium* plants than in vegetative plants (Khudairi and Bonde, 1954).

Roberts (1953) isolated another compound from a number of plants including *Xanthium*. This compound was found to have various antiauxin effects on plants (Roberts, 1953; Struckmeyer and Roberts, 1955; Roberts *et al.* 1957). This antiauxin appears to be chemically unrelated to xanthatin or xanthinin, since it has been identified as a 44-carbon, long-chain saturate Keto-alcohol (Struckmeyer and Roberts, 1955).

Although the possibility exists that the inhibitor reported in this work could be identical to one of the three previously isolated compounds discussed above, this does not seem likely. The three previously reported compounds each have very limited solubility in water at room temperature, whereas the inhibitor reported here was extracted in an aqueous system. Further work is being conducted in our laboratory to determine if the inhibitor reported here bears any relationship to the previously isolated compounds.

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